

**Abstract Submission No. : IL-9027**

**Th17 cells in acute kidney injury: Effects on injury, repair and progression**

David Basile

*Indiana University School of Medicine, United States*

Acute kidney injury (AKI) is a significant clinical concern which compromises kidney function due damage from ischemia or nephrotoxicity. While AKI is associated with high mortality rates, surviving patients typically experience recovery of renal function resulting from kidney repair responses. However, not all surviving patients recover full renal function and frequently many patients may transition to chronic kidney disease (CKD).

Work in our laboratory has focused primarily on vascular and immune pathways that contribute to injury and progression of CKD following AKI. While renal hypoxia may contribute to progressive renal disease, activation of immune responses also appear to modulate the efficacy of renal repair and subsequent progression of CKD. Previous studies from other investigators have nicely demonstrated that adoptive transfer of splenocytes from post-AKI mice promotes CKD in naïve recipient mice. Our laboratory developed a model of AKI-to-CKD transition in which rats are allowed to recover uneventfully from ischemia/reperfusion injury (I/R) for 5 weeks and subsequently challenged with elevated dietary salt for an additional 4 weeks. This maneuver results in dramatic renal inflammation, fibrosis and hypertension. Interestingly, rats are protected from this AKI to CKD transition when treated with the lymphocyte inhibitory agent, mycophenolate, during the high-salt diet phase.

Th-17 (CD4+/IL17+) cells are a type of effector T cell characterized by the production of the cytokine IL-17, which are often prominently induced in autoimmune disorders. We demonstrated that Th17 cells are the predominant T-effector cell type activated in the setting of AKI, and also are prominently re-expressed when rats were exposed to high-salt diet. In addition to Th17 cells, other immune cells such as NKT cells and CD8+ T-cells also contribute to the production of the cytokine IL-17 in the AKI to CKD transition. In addition, experimental maneuvers designed to inhibit IL17 activity support the hypothesis that Th17 cells promote the AKI-to-CKD transition. For example, treatment of rats with an IL17 receptor antagonist or an IL-17 immunoneutralizing antibody concurrent with elevated dietary salt was shown to attenuate the development of renal fibrosis, infiltration of macrophages, and neutrophils, and the degree of hypertension.

To further explore Th17 cells in AKI, a transgenic rat model was developed in which the *Rorc* gene, which codes for the major IL7 transcription factor ROR $\gamma$ T, was mutated with CRISPR/Cas9 technology. *Rorc*  $-/-$  rats manifest a reduced sensitivity to renal I/R injury relative to *Rorc*  $+/+$  rats, suggesting that Th17 cells contribute to early post ischemic damage. However, when ischemic time was increased in *Rorc*  $-/-$  to equalize the level of injury seen in *Rorc*  $+/+$  rats, mutant rats demonstrated increased mortality associated with a failed kidney repair response. Interestingly both adoptive transfer of *Rorc*  $+/+$  splenocytes and administration of rIL17 via osmotic minipump into post-I/R *Rorc*  $-/-$  rats increased survival and promoted renal recovery following I/R. These data suggest that Th17 cells play both beneficial and deleterious roles following acute injury, contributing to both early injury and late fibrosis, while also are contributing to tissue repair. The mechanism for this repair is currently unclear, but it may result in part by attenuating the degree magnitude of M1 macrophages.

Recently, we sought to understand the intracellular signaling pathways mediating IL17 expression in Th17 cells in AKI and CKD. Previous studies have demonstrated that the store-operated Ca<sup>2+</sup> channel Orai-1 may mediate lymphocyte calcium signaling during differentiation. In response to I/R, there is a substantial increase in Orai-1 expression in kidney CD4+ cells, which appears necessary for

expression of IL17. In vitro assays demonstrated that I/R injury-primed kidney lymphocytes increase cytosolic Ca<sup>2+</sup> and IL17 mRNA in response to various stimuli in vitro, and these activities are completely blocked by Orai-1 inhibitors. Moreover, Orai-1 inhibitors substantially decrease IL17 activation in vitro, and Th17 cell activation in both early AKI and the AKI-to-CKD transition in vivo. In addition, Orai-1+/CD4+ cells were found to be significantly increased in peripheral blood of patients after diagnosis of AKI in the ICU setting. Taken together, these data suggest that Th17 cells play an important role in the genesis of AKI and CKD transition, while blockade of Orai-1 may represent a potential therapeutic strategy to attenuate IL17 activation in patients with AKI.